Optimizing Transformation

The most characterized protocols we found are listed below.

Protocol 1

Protocol 1:

1) **Preparation**
   Aerobically grown M. magneticumAMB-1 was harvested and washed with 10 mM TES [N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] buffer containing 272 mM sucrose (pH 7.5) and resuspended in the same buffer at 10^9 cells/ml.
2) **Electroporation**
   A 50-µl cell suspension was aliquoted as electrocompetent cells. The cells were subjected to single-pulse electroporation and immediately transferred to 500 µl of Magnetic Spirillum growth medium (MSGM) supplemented with 20 mM Mg2+and incubated at 27°C overnight with shaking at 100 rpm.
3) **Plating**
   Cells were diluted in 5 ml of MSGM containing 0.7% agar and plated on 1% agar in MSGM containing 5 µg of ampicillin per ml or 2.5 µg of kanamycin per ml and incubated under anaerobic conditions.

Protocol 2

1) **Preparation**
   All manipulations were carried out on ice. For each pulsing experiment, 30 ml of a late-exponential phase culture (OD565=1, equivalent to about 10^10 cells) was harvested by centrifugation (14,000×g for 10 min). The cells were washed twice using an equal volume of electroporation buffer (1 mM HEPES, pH 7.0, 1 mM MgCl2, 200 mM sucrose) and resuspended to a final volume of 400 µl using the same buffer.
2) **Electroporation**
   An appropriate amount (0.2–5 µg) of plasmid DNA was added. After incubation for 5 min on ice, the mixture was loaded in a chilled 0.2-cm cuvette and subjected to a single pulse using a Gene pulser and a pulse controller apparatus (Biorad, Munich; settings: 1.2 kV, 600 Ohm, 25 µF). Pre-warmed liquid growth medium (4.5 ml) was added immediately, and the cells were incubated for 3 h at 28 °C for regeneration before they were plated onto ACA medium supplemented with the appropriate antibiotic.

To determine the optimal protocol for transformation, two separate cultures of AMB-1 were grown aerobically under the same conditions. It was determined that they had the same concentration of 10^-7 cells/mL. One of the cultures was transformed with Protocol 1 for one of the cultures and the other with Protocol 2. We found that only a very small pellet remained after addition of HEPES buffer outlined in the second protocol, while a sizable pellet form after addition of TES. This indicated that there were fewer cells in the tube for electroporation after HEPES was added.

After submitting the cells to electroporation (with no DNA added), we plated the recovered cells. We found that the cells subject to Protocol 1 settings produced colonies, while the cells subject to Protocol 2 did not, thereby indicating that the first is optimal.